

In re Application of:

Charles E. Prussak *et al.*

Application No.: 10/006,305

Filed: December 6, 2001

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PATENT

Attorney Docket No.: ST-UCSD3140

**In the Claims:**

The following listing of claims supersedes all prior listings of claims submitted in this application.

**Listing of Claims:**

1. (Cancelled)
2. (Previously Presented) A nucleic acid molecule encoding a chimeric TNF $\alpha$  ligand polypeptide having a CD154 Domain III and a TNF $\alpha$  Domain IV, wherein:
  - (a) the Domain III lacks a metalloproteinase cleavage site present in CD154; and,
  - (b) the Domain IV binds to a TNF receptor;wherein the encoded chimeric polypeptide is more resistant to cell membrane cleavage into soluble TNF $\alpha$  than are human pro-TNF $\alpha$  and human pro-TNF $\alpha$  lacking the TACE mmp recognition site spanning Val77 and Pro88 of human TNF $\alpha$ , when expressed in HeLa, 293, A549, COLO205, HCT15, BT20 or HT1080 cells.
3. (Previously Presented) The nucleic acid molecule of claim 2 further comprising a polynucleotide that encodes Domain II fragment of CD154.
4. (Previously Presented) The nucleic acid molecule of claims 2 or 3, further comprising a polynucleotide that encodes a Domain I fragment of CD154.
- 5-10. (Cancelled)

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11. (Previously Presented) The nucleic acid molecule of claim 2 further comprising a linker domain encoding a peptide of at least one amino acid that links the CD154 Domain III to the TNF $\alpha$  Domain IV.

12. (Previously Presented) The nucleic acid molecule of claim 2, comprising a nucleotide sequence consisting of SEQ.ID. NO. 1.

13-26. (Cancelled)

27. (Previously Presented) An expression vector, comprising the nucleic acid molecule of claim 2.

28. (Original) An expression vector, comprising the nucleic acid molecule of claim 3.

29. (Previously Presented) An expression vector, comprising the nucleic acid molecule of claim 4.

30-31. (Cancelled)

32. (Original) The expression vector of claim 27, further comprising viral DNA or bacterial DNA.

33. (Previously Presented) The expression vector of claim 32, wherein said viral DNA is selected from the group consisting of adenoviral DNA, retroviral DNA, or retroviral RNA.

34. (Previously Presented) The expression vector of claim 32, wherein at least a portion of the vector comprises adeno-associated viral DNA.

35. (Original) The expression vector of claim 27, further comprising a promoter region.

36. (Original) The expression vector of claim 27, further comprising a polyadenylation signal region.

37. (Previously Presented) A genetic construct comprising the nucleic acid molecule according to claim 2 operatively linked to a promoter sequence and to a polyadenylation signal sequence.

38. (Original) A host cell, comprising an expression vector according to claim 27 or a genetic construct according to claim 37.

39. (Original) The host cell of claim 38, wherein the cell is a mammalian cell.

40. (Original) The host cell of claim 39, wherein the cell is a tumor cell.

41. (Original) The host cell of claim 39, wherein the cell is an antigen presenting cell.

42-68. (Cancelled)

68. (Currently Amended) A process for producing a chimeric TNF $\alpha$  ligand polypeptide of claim 2 comprising culturing a host cell comprising an expression vector of claim 27 of claim 38 under conditions suitable to effect expression of the protein.

69-75. (Cancelled)

76. (Previously Presented) The nucleic acid molecule according to Claim 2, wherein the encoded chimeric polypeptide is about 90% less susceptible to cell membrane cleavage into

soluble TNF $\alpha$  than are native TNF $\alpha$  and TNF $\alpha$  lacking the metalloproteinase cleavage site present from Val77 to Pro88 of native TNF $\alpha$ .

77. (Previously Presented) An expression vector, comprising the nucleic acid molecule of Claim 76.

78. (Previously Presented) A genetic construct, comprising the nucleic acid molecule of Claim 76 operatively linked to a promoter sequence and to a polyadenylation signal sequence.

79. (Previously Presented) A host cell, comprising the expression vector of Claim 77 or the genetic construct of Claim 78.

80. (New) A process for producing a chimeric TNF $\alpha$  ligand polypeptide of claim 2 comprising culturing a host cell comprising a genetic construct of claim 37 under conditions suitable to effect expression of the protein.